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Continuous Air Embolization into Sheep Causes Sustained Pulmonary Hypertension and Increased Pulmonary Vasoreactivity

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Baseline pulmonary arterial, left atrial and systemic artery pressures, cardiac output, and lung lymph flow were measured in seven chronically catheterized sheep before continuous air embolization into the pulmonary artery, which caused a two-to-threefold increase in pulmonary vascular resistance (PVR) for 12 days. Air embolization was discontinued on days 4, 8, and 12 and hemodynamic measurements were repeated. Thromboxane B_2, 6-keto-$PGF_{1\alpha}$, and protein were measured in lung lymph and blood plasma on days 0, 4, 8 and 12. Air embolization caused an acute, sustained rise in pulmonary artery pressure and PVR (baseline, 3.68 ± 0.21; air, 8.32 ± 0.62, mean ± SE). By day 4, PVR was increased significantly even when air flow was interrupted (5.97 ± 0.72) and by day 12, it was almost twice baseline; pulmonary artery pressure also remained elevated (baseline, 19 ± 1 cm H_2O; day 12, 31 ± 3). Pulmonary vasoreactivity to PGH_2-A was significantly increased on days 4, 8, and 12 (day 12, 285 ± 41% of baseline response). Lung lymph flow, protein, and thromboxane clearance were increased throughout the study while clearance of 6-keto-$PGF_{1\alpha}$ was increased at day 4 and falling by day 8. At autopsy, morphometric analysis of the barium-injected pulmonary arterial bed revealed striking structural remodeling, extension of muscle into smaller arteries than normal: decreased peripheral arterial filling, increased medial thickness, and dilated large pulmonary arteries. Continuous air embolization into sheep causes the structural and functional changes of chronic pulmonary hypertension accompanied by increased pulmonary vasoreactivity to a bolus of PGH_2-A. The abrupt onset of the sustained elevation in PVR induced by air embolization may account for the severity of the structural remodelling, particularly for the increased medial thickness. (Am J Pathol 1988, 132:444–454)

CHRONIC PULMONARY hypertension has been found to develop as a secondary complication of acute lung injury, *eg*, ARDS and thromboembolism.[1-3] The mechanisms responsible for the sustained elevation of pulmonary artery pressure and the accompanying structural remodeling are not known but may include endothelial damage and inflammation.[4]

Infusion of air emboli into the pulmonary arterial bed of sheep has been shown to cause a rapid and sustained elevation in pulmonary artery pressure and pulmonary vascular resistance.[5] This response is accompanied by an increase in pulmonary vascular permeability and accumulation of neutrophils in the lung's microvasculature.[6] Repeated episodes of venous air embolization in rabbits have been shown to cause an increase in thickness of the muscular pulmonary arteries,[7] and in dogs, an increase in pulmonary artery pressure and some of the structural changes of pulmonary hypertension.[8]

The present study addresses the question of whether continuous air embolization into the pulmonary arterial bed of sheep causes the structural and functional changes of sustained pulmonary hypertension and alterations in pulmonary vasoresponsiveness. Air emboli were infused continuously into the pulmonary ar-

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tery of chronically instrumented sheep over a 12 day period. On days 4, 8, and 12, the embolization was interrupted and, after 1 hour of equilibration, pulmonary vascular pressures and vasoreactivity were monitored. Continuous air embolization caused the gradual onset of sustained pulmonary hypertension associated with an increased pulmonary pressor response to a bolus injection of the potent pulmonary vasoconstrictor PGH_2-A. Morphometric analysis of lung tissue at autopsy revealed structural changes typical of chronic pulmonary hypertension. The severity of the pulmonary hypertension and its rapidity of onset in comparison with other sheep models make this an ideal model in which to study the pathogenesis of this disease.

Materials and Methods

Animal Preparation

Chronically instrumented sheep were prepared as described previously.[9] Thirteen mixed-breed sheep (30 to 35 kg body weight) had bilateral thoracotomies for placement of pulmonary arterial and left atrial catheters and for cannulation of the efferent lymph duct of the caudal mediastinal node. Carotid artery, jugular venous, and Swan-Ganz catheters were placed through an incision in the neck. The sheep were kept in cages with free access to food and water. The experiments were begun after a 5 to 7 day recovery period.

Physiologic Measurements

Pulmonary arterial, left atrial, and systemic arterial pressures were measured continuously and recorded every 15 minutes during monitoring periods. Cardiac output was measured every 30 minutes using an Edwards model 9520A Thermodilution Cardiac Output Computer (American Edwards Laboratories, Irvine, CA). Pulmonary vascular resistance was calculated by subtracting the left atrial pressure from the pulmonary artery pressure and dividing by cardiac output.

Pulmonary Vasoreactivity

Animals breathed 100% oxygen for 5 minutes. Vascular pressures, cardiac output and arterial blood gases were measured. After the physiologic parameters returned to baseline, the animals breathed 12% oxygen for 5 minutes and the same measurements were made. At all times studied, 100% oxygen caused an arterial PO_2 of greater than 300 torr and 12% O_2 caused an arterial PO_2 of below 50 torr.

Pulmonary vasoreactivity to PGH_2-A then was assessed. After returning to baseline, the animal was given a bolus injection of PGH_2-A (12.5 μg in 1 ml of saline) into the pulmonary artery and 30 seconds later vascular pressures were recorded and cardiac output was measured. When the animal had returned to baseline a second dose (25 μg in 1 ml) was given and the response was recorded again. The doses were determined from previous studies and were selected from the range that caused a doubling of the pulmonary artery pressure in control animals.

Blood and Lung Lymph Measurements

Arterial blood samples were taken (two samples over a 1-hour period) for measurement of pH, blood gases, hematocrit, leukocyte counts and total serum protein. Lung lymph flow was recorded every 15 minutes and samples were pooled every 30 minutes. The blood and lymph samples were centrifuged and protein concentrations were measured in plasma and lung lymph with an AutoAnalyzer (Technicon Instruments) using a modified Biuret method.[10] In addition, 6-keto-$PGF_{1\alpha}$ and thromboxane B_2, the stable metabolites of prostacyclin and thromboxane A_2, respectively, were measured in lung lymph and plasma by radioimmunoassay. Both of these assays have been described previously[11]; the detection limit of both assays is less than 20 pg/ml.

Experimental Protocol

Physiologic measurements, assessment of pulmonary vasoreactivity, and collection of lung lymph and blood were made at baseline on 2 separate days before start of chronic air embolization. Seven animals received continuous air embolization. In these animals, filtered room air was continuously infused into the right atrium through a Swan-Ganz catheter. The air was infused at a rate sufficient to cause a two-to-threefold increase in pulmonary vascular resistance. The initial rate of infusion was determined over a 4-hour period and was 0.04 ml/kg/min, but over the course of the experiment the rate required to maintain the increase in pulmonary vascular resistance fell (final rate, 0.02 ml/kg/min). Five animals that did not receive embolization served as controls.

Assessment of the Effect of Chronic Air Embolization

The sustained effect of air embolization on the pulmonary vascular bed was assessed on days 4, 8, and

12. Initially, physiologic monitoring, including lung lymph and blood measurements, were followed over a 90-minute period. These results are reported below as the "on air" data. Air embolization was then stopped and after a 1-hour period, physiologic monitoring and blood gases were repeated and pulmonary vasoreactivity was assessed. These results are the "off air" data. After completion of monitoring, air embolization was resumed. The animals were killed with an overdose of barbiturate at the end of the experiment.

Structural Studies

Biopsy Tissue

Lung biopsy tissue was obtained at the time of the initial surgery and at autopsy as described previously.[12] The lungs were inflated to a pressure of 35 cm H_2O and a peripheral portion of the lung was clamped; a second clamp was placed more proximally and the lung was incised between the clamps. The cut edge of the lung was oversewn with a continuous suture. The biopsy tissue was immersed in 10% formol-saline overnight and the clamp was removed. The tissue was cut into three pieces and processed for routine light microscopy of hematoxylin and eosin-stained (H&E) 5-μ sections. The number of granulocytes and alveolar profiles were counted in 10 (×40 objective) consecutive fields containing alveoli only.[13]

Autopsy Tissue

At autopsy the heart and lungs were removed intact. The left pulmonary artery was cannulated and the pulmonary arterial circulation was perfused for 5 minutes with a barium sulfate-gelatin mixture from a pressure of 100 mm Hg.[12] The trachea was then cannulated and the airways were inflated with formol-saline from a pressure of 25 cm H_2O. The entire heart and lungs were immersed in formal-saline for fixation.

After 1 week of fixation, arteriograms were made of the barium-injected pulmonary circulation. The first major branch of the pulmonary artery was identified on the arteriogram and its lumenal diameter was measured at the origin of the branch and at distances of 25 and 50% along its length traveling towards the periphery. The diameter of the terminal intraacinar vessels was also measured. The density of background haze, arteries too small to be distinguished as individual lines, was assessed also. In addition, an arteriogram was taken of a 1-cm slice through the mid-region of each lung from apex. This arteriogram allowed more accurate assessment of peripheral arterial filling.

Random blocks were taken from all lobes and processed for routine light microscopy. Two 5-μ thick sections were cut from each block; one was stained with H&E and the other with a Miller's elastic stain followed by a Van Gieson. Using quantitative techniques,[12] these sections then were examined for the structural changes of chronic pulmonary hypertension. At least 100 consecutive arteries were examined from each animal (25 arteries from each of 4 blocks). Sections showing large areas of nonfilled vasculature were not used for morphometric assessment. Barium-filled arteries were characterized by structure (muscular, partially muscular, or nonmuscular), and the arteries were landmarked by the accompanying airway (bronchus, bronchiolus, terminal bronchiolus, respiratory bronchiolus, alveolar duct, and alveolar wall). The external diameter and the medial thickness of each artery were also measured and percent medial thickness was calculated using the formula: medial thickness ×2/external diameter ×100. For evaluation of the volume of the peripheral arterial bed, the number of alveolar profiles and barium-filled arteries were counted in 25 consecutive fields containing alveolar tissue only.

Right ventricular hypertrophy was assessed after fixation. The heart was separated from the lungs and the atria were removed. The right ventricle (RV) was then dissected from the heart and the RV and the remaining left ventricle plus septum (LV + S) were weighed separately. RV weight was expressed as the ratio of the right ventricle to the left ventricle plus septum (RV/LV + S).

Statistical Analysis

For each animal, the mean and standard error of the mean (m ± SE) was calculated for all variables. Baseline values were taken as the mean of the 2 days of baseline monitoring. Pulmonary vasoreactivity was expressed as percent of baseline response. Data from the experimental animals were compared to controls using analysis of variance and covariance with repeated measures.[15] Nonparametric data were compared using the Wilcoxon sign-rank test. Structural data were analyzed using an unpaired *t*-test or chi square analysis. A P value of less 0.05 was considered significant.

Results

During Air Infusion (On Air)

Physiological Measurements

Continuous air embolization caused an immediate and sustained increase in both pulmonary vascular re-

Table 1—Hemodynamic and Blood Data From Experimental Animals During Air Embolization

	Days of air emobilization			
	0 (N = 7)	4 (N = 7)	8 (N = 7)	12 (N = 5)*
Hemodynamics				
PVR	3.68 ± 0.21	8.32 ± 0.62†	8.05 ± 0.35†	8.79 ± 0.82†
P_{pa} (cm H_2O)	19 ± 1	34 ± 2†	34 ± 2†	39 ± 3†
P_{la} (cm H_2O)	−1 ± 1	−5 ± 1†	−4 ± 1†	−1 ± 1
CO (l/min)	5.41 ± 0.30	4.54 ± 0.28	4.69 ± 0.33	4.33 ± 0.28
P_{sa} (mm Hg)	87 ± 6	84 ± 7	81 ± 6	82 ± 7
Blood				
PO_2 (Torr)	93 ± 2	66 ± 3†	63 ± 3†	65 ± 1†
PCO_2 (Torr)	34 ± 1	36 ± 1	36 ± 1	31 ± 2
pH	7.53 ± 0.01	7.51 ± 0.01	7.50 ± 0.01	7.53 ± 0.02
Leukocytes per cu mm	6860 ± 757	6764 ± 688	6071 ± 607	7483 ± 1309
Hematocrit (%)	31 ± 3	33 ± 3	35 ± 4	34 ± 2

Data are presented as mean ± SE.

* Two animals were sacrificed before the 12th day because of catheter problems.

† $P < 0.05$ as compared with baseline.

PVR, pulmonary vascular resistance; P_{pa}, mean pulmonary arterial pressure; P_{la}, left atrial pressure; CO, cardiac output; P_{sa}, mean systemic arterial pressure.

sistance and pulmonary artery pressure (Table 1). Continuous air embolization also resulted in a small but significant decrease in left atrial pressure on days 4 and 8, but cardiac output and systemic artery pressure remained similar to baseline levels (Table 1).

Blood Gases and Lung Lymph Measurements

Continuous air embolization caused a significant 30% reduction in arterial PO_2 throughout the 12 days of the study; arterial PCO_2 and pH, blood leukocyte count, and hematocrit remained within the normal range (Table 1). Continuous air embolization also resulted in a sustained and significant two-to-threefold increase in lung lymph flow and in lung lymph protein clearance, although the lymph to plasma protein concentration ratio remained at baseline values throughout the study (Table 2).

Although there was no statistical difference in absolute levels of lung lymph prostacyclin and thromboxane (Table 2) in the air-embolized animals over the period of study, lung lymph clearance of these two prostanoids was significantly increased on days 4 and 8 (Figure 1). Lung lymph thromboxane clearance was elevated more strikingly than that of prostacyclin on days 8 and 12. Plasma levels of thromboxane B_2 and 6-keto $PGF_{1\alpha}$ were unchanged throughout the course of the study (data not shown).

There was no significant variation for any measured variable in the control animals over the 12 days of the study (data not shown). These values were similar to the baselines shown in Tables 1 and 2 for the experimental group before receiving continuous air embolization.

During Interruption of Air Infusion (Off Air)

Physiological Measurements

Continuous air embolization caused development of sustained pulmonary hypertension which persisted

Table 2—Lymph Data From Air-Emobilized Sheep

	Days of emobilization			
	0 (N = 5)	4 (N = 5)	8 (N = 5)	12 (N = 5)*
Lymph flow ml/15 min	1.3 ± 0.3	4.2 ± 0.8†	3.9 ± 0.9†	3.7 ± 0.6†
Protein lymph/plasma	0.64 ± 0.03	0.71 ± 0.03	0.66 ± 0.04	0.63 ± 0.05
Protein clearance (ml/15 min)	0.80 ± 0.17	2.92 ± 0.48†	2.50 ± 0.54†	2.29 ± 0.29†
Thromboxane B_2 (ng/ml)	0.23 ± 0.05	0.30 ± 0.07	0.29 ± 0.08	0.25 ± 0.15
6-keto $PGF_{1\alpha}$ (ng/ml)	0.05 ± 0.01	0.59 ± 0.40	0.13 ± 0.06	0.04 ± 0.01

Data are presented as mean ± SE.

* Prostanoid measurements were not available for two sheep.

† $P < 0.05$ when compared with baseline.

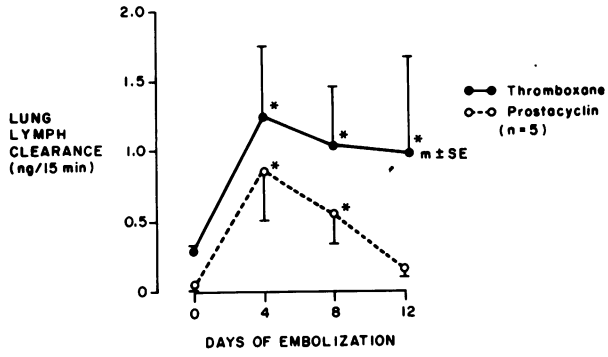

Figure 1—Lung lymph thromboxane and prostacyclin clearance during 12 days of continuous air embolization. The fall in lung lymph prostacyclin clearance coincides with the onset of sustained pulmonary hypertension.

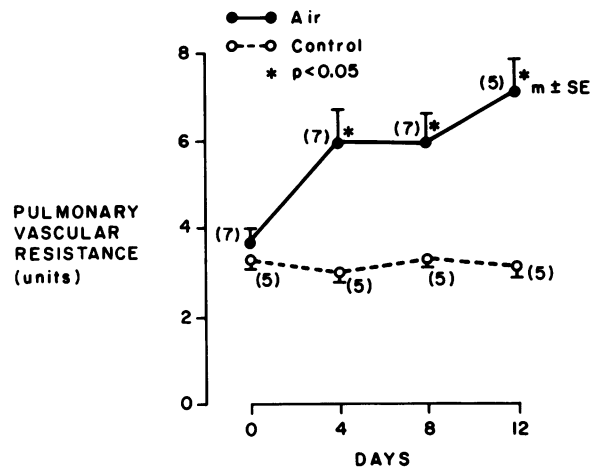

Figure 2—Pulmonary vascular resistance over a 12-day period in sheep that had received continuous air embolization. The measurements were made after the air embolization had been interrupted for a 1-hour period. Data from control sheep are shown for comparison (number of animals).

when air embolization was interrupted (Table 3). The severity of the hypertension increased over the 12 days of the study (Figure 2). Pulmonary vascular resistance was significantly elevated from day 4, and pulmonary artery pressure from day 8 (Table 3).

Blood Gases

Even when off air, arterial PO_2 in the embolized animals remained significantly lower than baseline but arterial pH and PCO_2 remained within the normal range (Table 3).

Pulmonary Vasoreactivity

When challenged with PGH_2-A, the embolized animals showed a significant increase in pulmonary vasoreactivity over baseline response. After 4 days of embolization the animals showed a 50% increase in pulmonary vascular resistance, increasing to a nearly threefold increase above baseline by 12 days (Figure 3). The pulmonary artery pressor response to PGH_2-A increased significantly by day 4 (140% of baseline response). Cardiac output showed a small but insignificant decrease. Control animals maintained consistent responses to PGH_2-A throughout the experimental period (Figure 3).

Breathing 12% oxygen caused a 7 ± 1 cm H_2O increase in baseline pulmonary artery pressure. On day 8, four of the experimental animals had an exaggerated hypoxic pressor response (312 ± 67% of baseline response), but at all other times (4 and 12 days) the animals' response was similar to baseline. The hypoxic pressor response in control animals did not change over the course of the study (data not shown).

Breathing 100% oxygen resulted in a 2-cm H_2O decrease in pulmonary artery pressure at baseline. This response did not change over the course of the experi-

Table 3—Hemodynamic and Blood Gas Data for Embolized Sheep During Interruption of Air Infusion (Off Air)

	Days of air emobolization			
	0 (N = 7)	4 (N = 7)	8 (N = 7)	12 (N = 5)*
Hemodynamics				
PVR	3.68 ± 0.21	5.97 ± 0.72†	5.90 ± 0.63†	7.04 ± 0.69†
P_{pa} (cm H_2O)	19 ± 1	21 ± 1	25 ± 2†	31 ± 3†
P_{la} (cm H_2O)	−1 ± 1	−4 ± 1	−2 ± 1	−3 ± 1
CO (l/min)	5.41 ± 0.30	4.10 ± 0.36	4.36 ± 0.40	4.35 ± 0.43
Blood				
PO_2 (torr)	93 ± 2	71 ± 4†	70 ± 3†	73 ± 6†
PCO_2 (torr)	34 ± 1	33 ± 1	32 ± 1	30 ± 2
pH	7.53 ± 0.01	7.52 ± 0.02	7.51 ± 0.01	7.53 ± 0.01

Data are presented as mean ± SE.

* Two animals were sacrificed before the 12th day because of catheter problems.

† $P < 0.05$ when compared with controls.

PVR, pulmonary vascular resistance; P_{pa}, mean pulmonary arterial pressure; P_{la}, left atrial pressure; CO, cardiac output; P_{sa}, mean systemic arterial pressure.

ment in either air embolized or control animals (data not shown).

Structural Data

Gross Examination

At autopsy, the lungs from the embolized animals showed areas of consolidation and infarction, predominantly in the posterior regions of the lower lobes.

Arteriograms

Arteriograms of the lungs of the embolized animals showed marked dilatation of the large pulmonary arterial branches and abrupt tapering in the peripheral branches (Figure 4). Arterial diameter along the first branch from the axial artery showed a significant increase in lumen diameter (Figure 5). Arteriograms of the 1-cm slices showed a marked and patchy reduction in background haze, reflecting a decrease in the number of small barium-filled arteries (Figure 4).

Light Microscopic Examination

Although structural changes were present in all sections from all embolized animals, there were localized variations in severity. The majority of embolized animals showed thickening of the alveolar walls due to both appearance of edema and a mononuclear cell infiltrate. In sections from all embolized animals, areas were found where the pulmonary arteries were markedly dilated, as noted in the arteriograms. Other areas showed patent pulmonary arteries whose lumens contained no barium and still other areas, contained arteries whose lumens were completely occluded by either a cellular plug (Figure 6A) or acellular, amorphous material. Recanalized arteries were also a feature (Figure 6B). The veins in the embolized lungs appeared grossly normal while the bronchial circulation, particularly in the areas of arterial occlusion appeared more obvious than normal. In one lung from an embolized animal, the venous side of the circulation was injected and the venous pattern of barium filling was found to be normal, but bronchial artery filling was prominent. Counts of the number of granulocytes per 100 alveolar profiles showed no increase above baseline in either the embolized or control animals (baseline, 9.0 ± 1.6 granulocytes per 100 alveoli; 12-day control, 9.7 ± 1.3; 12 days of embolization, 7.9 ± 1.1).

Morphometric Assessment of Chronic Pulmonary Hypertension

Continuous air embolization caused significant appearance of muscle in the normally nonmuscular arterial walls at all intraacinar levels—respiratory bronchiolar, alveolar duct and alveolar wall (Table 4 and Figure 7). Medial thickness of the normally muscular arteries of all sizes showed a significant twofold increase above control values (Figures 7 and 8). The adventitia of many of these arteries was also increased in thickness and included layers of elastin (Figure 8) that were not seen in the control animals. Massons's trichrome stain showed the presence of increased proteoglycans in the thickened media and adventitia of these arteries; the intima often showed eccentric intimal hyperplasia.

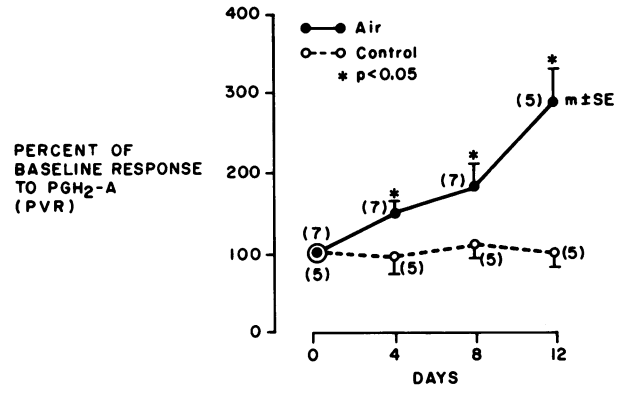

Figure 3—Pulmonary vascular response to a bolus injection of PGH_2-A in sheep over the 12-day period of continuous air embolization. The measurements were made after air embolization had been stopped for 1 hour. Data are presented as percent of the baseline increase in pulmonary vascular resistance. The responses of control sheep are shown for comparison (number of animals).

Continuous air embolization, caused a significant 50% reduction in the number of barium-filled arteries per 100 alveolar profiles (Table 5). The number of filled arteries in Table 5 represents an underestimate, because sections with no barium filling were not included in the counts of barium-filled arteries.

Although the right ventricle appeared dilated in all embolized sheep, RV/LV + S of the air embolized animals was not significantly increased over that of the control animals (controls, RV/LV + S = 0.32 ± 0.01; air = 0.38 ± 0.06).

Discussion

Continuous air embolization of the pulmonary arterial bed in sheep causes development of sustained pulmonary hypertension and increased pulmonary vasoreactivity. Pulmonary vascular resistance is increased by day 4, and by day 8 pulmonary artery pressure is elevated. These physiologic changes are accompanied by structural remodeling of the pulmonary arteries including appearance of muscle in normally

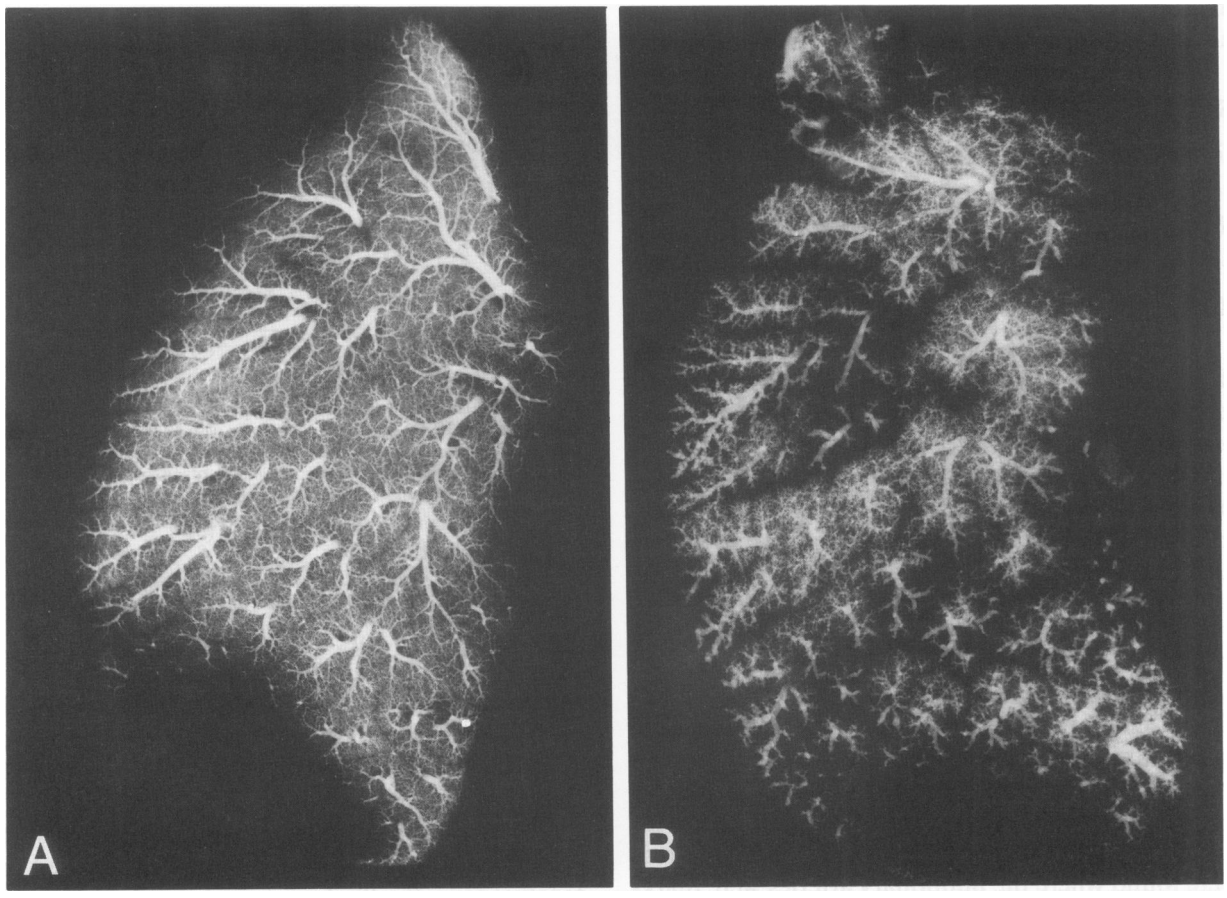

Figure 4—Arteriograms of 1-cm slices of lung from control lung showing normal luminal filling of the arterial tree with barium (**A**) and from a lung after 12 days of continuous air embolization showing dilatation of some arteries, particularly towards the pleural surface, and patchy loss of barium filling (**B**). ×0.5

nonmuscular intra-acinar arteries, decreased number of barium-filled arteries, arterial dilatation, and increased medial thickness of normally muscular arteries.

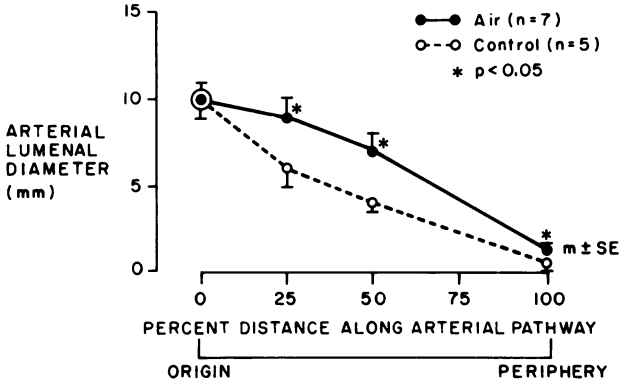

Figure 5—Arterial lumenal diameters along the first major arterial branch of the pulmonary artery from sheep following 12 days of continuous air embolization and from control sheep. Measurements were made from whole lung arteriograms in which the pulmonary arterial bed was injected with barium.

Chronic pulmonary hypertension has been induced previously in sheep by repeated infusions of endotoxin[16] or indomethacin,[12] or after thoracic irradiation.[17] In those models, however, the development of the hypertension was gradual, whereas with air embolization, it is immediate. The rapid, severe, and sustained nature of the elevation in pulmonary artery pressure and pulmonary vascular resistance caused by continuous air embolization may contribute to the more striking structural changes seen in this model.

In previous sheep models of chronic pulmonary hypertension,[12,16,17] extension of muscle into intraacinar arteries and reduction in number of barium-filled peripheral arteries have been identified consistently, but an increase in medial thickness of the normally muscular arteries has not been demonstrated. However, in those models of chronic pulmonary hypertension the elevations in pulmonary artery pressure and pulmonary vascular resistance were not as severe as seen in the present study. This suggests that for medial thickening to occur, a sustained and marked increase in

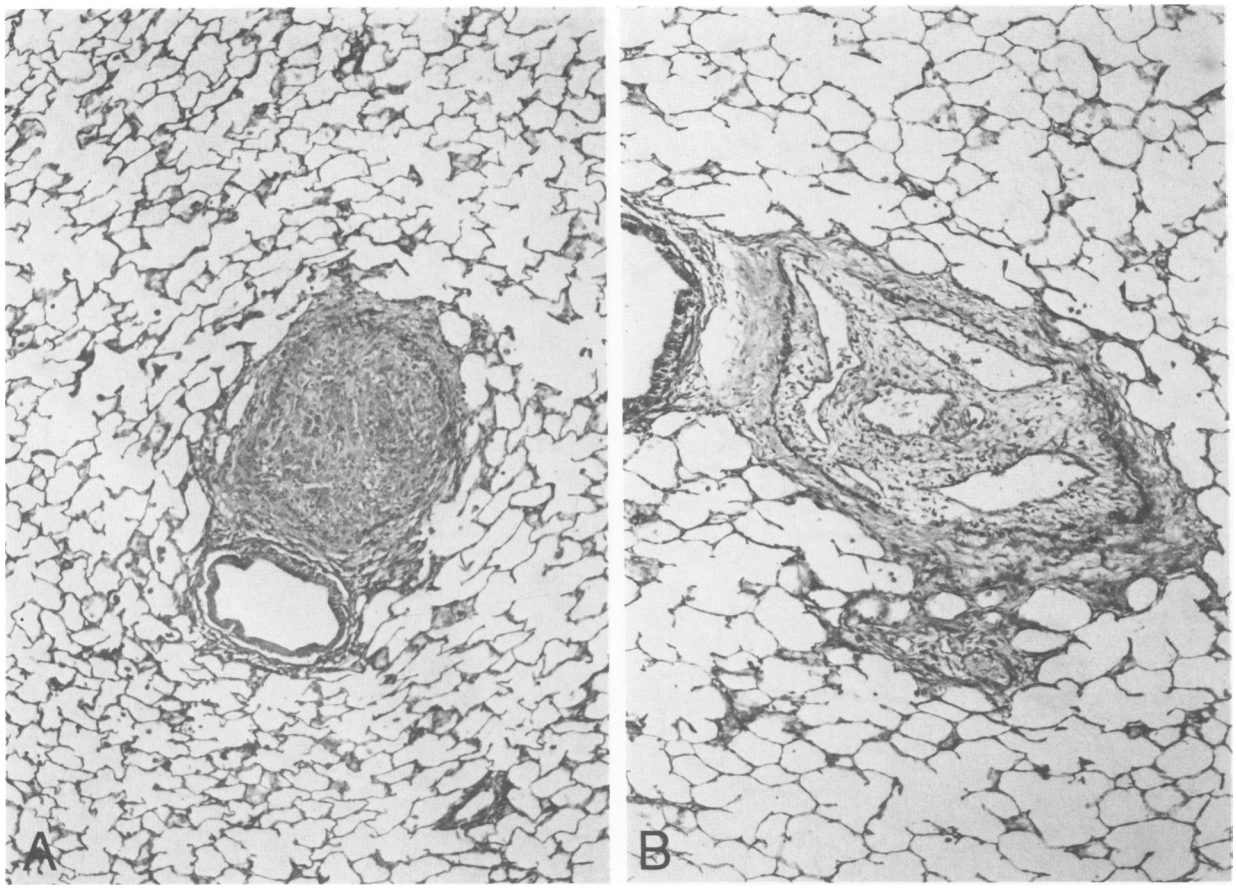

Figure 6—Light micrographs from lungs after 12 days of continuous air embolization showing an artery completely occluded by cells and amorphous debris (**A**) and a recanalized artery (**B**). Elastic Van Gieson, ×125

pulmonary artery pressure and pulmonary vascular resistance is required. These data also support the idea that the medial thickening is secondary to elevations in pulmonary artery pressure and resistance.[18]

Clinical and experimental studies also indicate that in certain situations the severity of the initial rise in pulmonary artery pressure or pulmonary vascular resistance may portend the subsequent development of sustained pulmonary hypertension. For example, follow-up of patients with recurrent or occult pulmonary embolism revealed that only patients with pulmonary artery pressures greater than 30 mm Hg on their first evaluation progressed to severe pulmonary hypertension and death.[2] Furthermore, follow-up of dogs 10 to 23 months after a single embolus of starch to one lung showed that dogs with a greater than 50% rise in pulmonary artery pressure within 1 hour of embolization were more likely to develop sustained pulmonary hypertension than dogs who did not have a marked acute response.[19]

Structural remodeling of the pulmonary arterial tree contributes to the maintenance of chronic pulmonary hypertension seen from day 8 of embolization.[18] In the embolized animals, prolonged hypoxic vasoconstriction is unlikely to contribute to the hypertension because breathing 100% oxygen did not reverse the sustained hypertension and the arterial PO_2 was above 60 torr. However, the transient increased

Table 4—Number of Muscular, Partially Muscular, and Nonmuscular Arteries at Intra-acinar Airway Levels

	Number of arteries		
	Air	Control	
Respiratory bronchiolus			
Muscular	128	69	chi sq = 13.05
Partially muscular	32	28	$P \leq 0.05$
Nonmuscular	15	27	
Alveolar duct			
Muscular	61	15	chi sq = 28.86
Partially muscular	58	44	$P \leq 0.05$
Nonmuscular	70	92	
Alveolar wall			
Muscular	26	–	chi sq = 52.29
Partially muscular	39	–	$P \leq 0.05$
Nonmuscular	130	125	

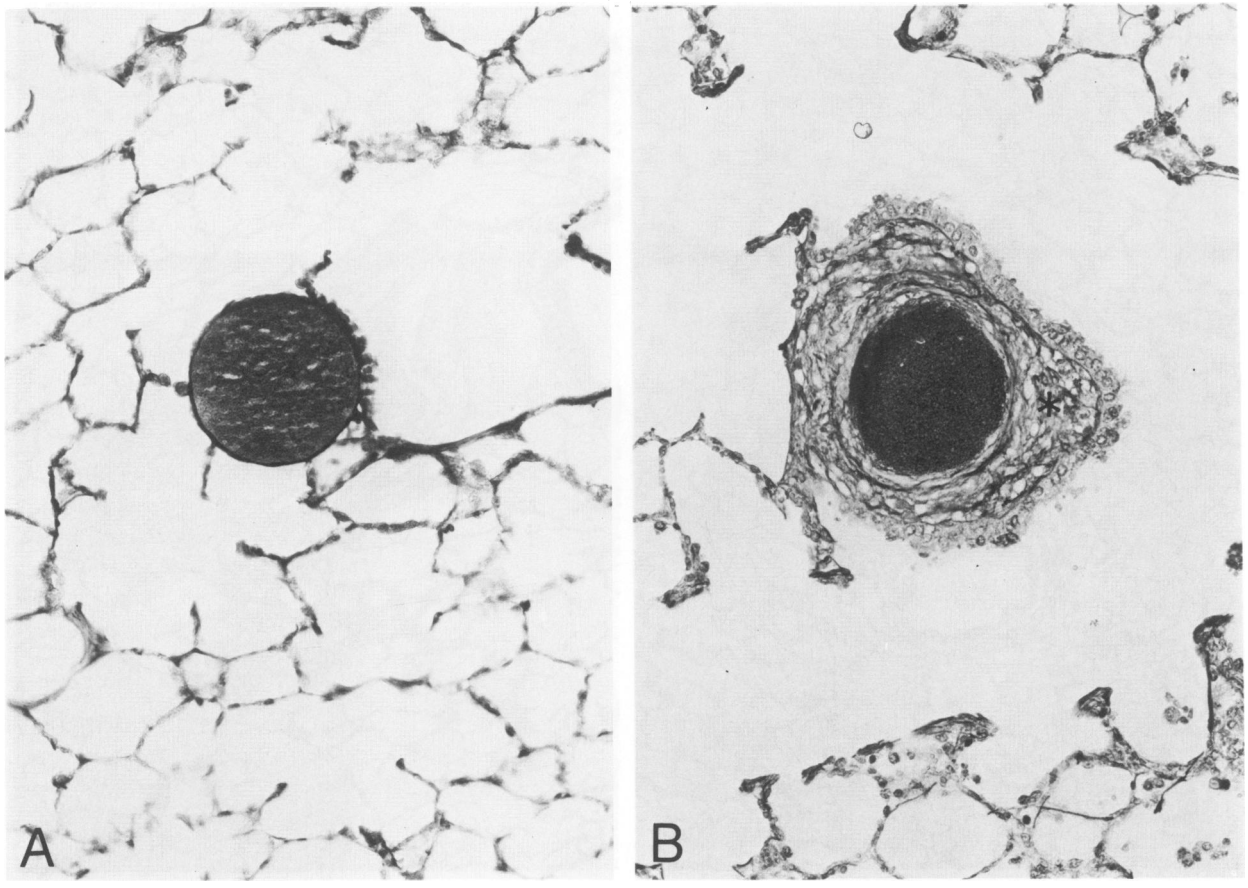

Figure 7—Light micrographs of barium-filled arteries at respiratory bronchiolar level. **A**—a nonmuscular artery from a control and **B**—a muscular artery with thickened media and adventitia from an animal that received 12 days of continuous air embolization. The adventitia contains numerous layers of elastin (*). Elastic Van Gieson, ×320

hypoxic pressor response seen in four embolized animals might suggest that the animals go through a period when this heightened sensitivity to hypoxia could result in episodes of vasoconstriction. Other stimuli that lead to the development of sustained pulmonary hypertension induced by continuous air embolization may include endothelial cell injury, edema, inflammation, alterations in vasoactive mediators, and restriction of the vascular bed by vasoconstriction and/or obstruction. Further studies will be needed to explore these mechanisms.

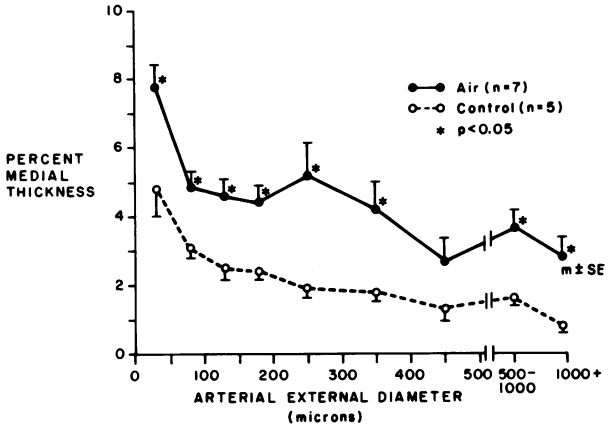

Figure 8—Percent medial thickness for muscular arteries of various external diameters in sheep following 12 days of continuous air embolization and in control sheep.

Lung lymph flow and lung lymph protein clearance were elevated in the air-embolized sheep throughout the study, suggesting a sustained increase in pulmonary microvascular permeability. Increased lung lymph flow may result from increased driving pressure for fluid filtration, but in that case lymph/plasma protein concentration falls.[20,21] In the air-embolized sheep, lymph flow increased without a decrease in lymph/protein concentration. The increase in lung lymph flow without a decrease in lymph/plasma protein concentration could be explained by increased microvascular surface area, but in air embolized sheep the marked reduction in the number of barium-filled

arteries suggests a decreased microvascular surface area. Thus, pulmonary edema, and presumably endothelial injury, could contribute to the development of chronic pulmonary hypertension after continuous air embolization.

Inflammation has been suggested as a stimulus to the development of chronic pulmonary hypertension.[4] Air embolization results in early sequestration of granulocytes in peripheral lung, a phenomenon that has been linked to the development of increased pulmonary vascular permeability. Structural examination of the lung shows granulocyte accumulation around the air bubbles within hours of initiation of the embolization,[6] and granulocyte depletion with nitrogen mustard attenuates the rise in pulmonary vascular permeability.[22] Histologic examination of biopsy tissue obtained after 12 days of air embolization failed to show any accumulation of granulocytes in lung periphery, but whether the early accumulation of granulocytes in this model contributes to the pathogenesis of chronic pulmonary hypertension is not certain.

In the normal lung, circulating humoral mediators, particularly prostanoids, can induce vasoconstriction. Continuous air embolization resulted in a sustained increase in lung lymph thromboxane clearance and only a transient increase in that of prostacyclin. Temporally, the increase in thromboxane/prostacyclin ratio paralleled the onset of sustained pulmonary hypertension, suggesting a link between increased levels of the pulmonary vasoconstrictor prostanoid, thromboxane A_2, and the development of chronic pulmonary hypertension.

The source of thromboxane in lung lymph is not certain but peripheral blood mononuclear cells and a recently described cell type in sheep, goat, and pig lung, the pulmonary intravascular macrophage[23,24,25] (activated monocyte), may be responsible for production of this prostanoid. In sheep, at least, platelets are not a rich source of thromboxane.[26] Whether the pulmonary intravascular macrophage is activated or increased in number by air embolization is not yet known but structural studies are currently being carried out in the authors' laboratories.

The increased pulmonary pressor response to PGH_2-A in the air embolized animals could also reflect an imbalance between circulating vasoconstrictor and vasodilator agents. Alternatively, structural remodeling of the pulmonary vascular bed or endothelial damage could play a role. While medial thickening of muscular arteries in the embolized animals may contribute to the increased pressor response to PGH_2-A, the striking reduction in patent peripheral arterial bed is likely to be the most important consideration.

Table 5—Alveolar and Arterial Number in Air-Embolized and Control Sheep Lungs

	Alveoli per unit area	Arteries per unit area	Arteries per 100 alveoli
Air (N = 7)	46.8 ± 2.6	0.8 ± 0.1	1.86 ± 0.10*
Control (N = 5)	47.6 ± 2.8	1.9 ± 0.1	3.73 ± 0.26

Data are given as m ± SE; unit area = 0.13 sq mm.
* $P < 0.05$.

Dilatation of the large pulmonary arteries and bronchopulmonary arterial shunts also were found in the animals receiving continuous air embolization. The pulmonary arterial dilatation is perhaps not surprising in view of the loss of peripheral vascular bed, and probably reflects the increase blood volume in arteries proximal to the obstruction. Dilatation of large pulmonary arteries is seen in patients with chronic pulmonary hypertension. Recent experimental studies have shown marked hypertrophy of the bronchial circulation after occlusion of a major pulmonary artery.[27] Thus, the barium-filling of bronchial circulation in the embolized animals is consistent with pulmonary artery occlusion. Such occlusion was apparent in the arteriograms.

In summary, it has been shown that continuous air embolization of the pulmonary arterial bed of the sheep leads to the development of chronic pulmonary hypertension accompanied by an increase in pulmonary vasoresponsiveness. Of the models of chronic pulmonary hypertension in sheep described thus far, the air embolization model results in the most severe elevation in pulmonary vascular resistance and in the most pronounced structural remodeling. The structural changes include extension of muscle into smaller arteries than normal and reduction in peripheral arterial volume, as well as increased medial thickness of muscular arteries. These findings are consistent with a link between the level of pulmonary artery pressure and the development of medial thickening, and suggest that the latter occurs only when pulmonary artery pressure and resistance are maintained at a level that is approximately twice baseline.

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